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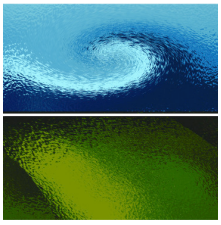

RESEARCH ARTICLE

The co-occurrence of *Callinectes sapidus* Rathbun, 1896 (Brachyura: Portunidae) and the parasitic dinoflagellate *Hematodinium* sp. (Dinoflagellata: Syndinidae) in two transitional water ecosystems of the Apulia coastline (South-Italy).

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Abstract

1 - Coastal marine and transitional water ecosystems are threatened world-wide by invasions of non-indigenous invertebrate species, altering community structure and ecosystem functioning.

2 - Here, we report on the occurrence of the Atlantic blue crab *Callinectes sapidus* in transitional water ecosystems of the Apulia coastline (Salento peninsula, SE Italy).

3 - Blue crabs were sampled seasonally, every three months over a whole-year period in the Torre Colimena basin (Ionian Sea) and Acquatina lagoon (Adriatic Sea). Specimens were identified and enumerated. In addition, their hemolymph was screened for parasite infections using histological techniques.

4 - *Callinectes sapidus* was sampled in the Torre Colimena basin, where no other crab species were collected during the whole experimental period. In contrast, in the Acquatina Lagoon four crab species were sampled, including *C. sapidus*.

5 - The parasitic dinoflagellate *Hematodinium* sp., known to determine in *C. sapidus* the degenerative "bitter crab syndrome" in its native habitats, was ubiquitously detected in the hemolymph of blue crabs from both habitats and in autochthonous brachyurans collected in the Acquatina Lagoon.

6 - An assessment of the diverse spectrum of potential ecological effects triggered by the *Callinectes-Hematodinium* interaction on autochthonous brachyuran species is presented, together with a preliminary evaluation of the molecular approaches to be implemented in order to verify the species-specificity of the host-parasite interaction.

Keywords: *Callinectes sapidus;* not native species; *Hematodinium* sp.; bitter crab syndrome; host-parasite interaction; ecological impact; transitional water ecosystems; Salento peninsula.

Introduction

Originating from western Atlantic, the blue crab *Callinectes sapidus* Rathbun, 1896 (Decapoda, Brachyura, Portunidae) has to date extended its distribution in most European coastal waters and in Japan (Nehring, 2011). In Europe, the first record of *Callinectes* dates back to 1900 on the Atlantic coast of France. Subsequently, specimens were detected in the North Sea (1932), Mediterranean Sea (1949, but probably as early as 1935), Baltic Sea (1951), Black Sea (1967), and possibly in the Sea of Azov (1967). In the Mediterranean Sea the species was first reported in Israel in

1955 (Holthuis and Gottlieb, 1955); in Italy it was observed in the Northern Adriatic Sea (Giordani Soika, 1951) and in the Genova Gulf (Tortonese, 1965). The majority of the reports on the blue crab in Mediterranean and, in general, European waters refers to episodic catches, limited in the number of specimens collected and the temporal period considered (Dulčić *et al*., 2010; Nehring, 2011 and literature cited; but see Tureli Bilen *et al*., 2011; Carrozzo *et al*., 2013). In addition, virtually no information is available on the functional role played by this species within benthic food webs of Mediterranean coastal systems.

In its native habitats, *C. sapidus* represents an important component of estuarine communities (Baird and Ulanowicz, 1989; Dittel *et al*., 2000). It has omnivorous, opportunistic trophic habits, feeding on plants, invertebrates, carcasses and conspecifics (Kennedy and Cronin, 2007); natural controls upon blue crab populations are represented by a number of large-sized fish predators including drums (e.g., *Pogonias cromis* and *Sciaenops ocellatus*), gars (e.g., *Lepisosteus spatula* and *L. oculatus*) and catfishes (e.g., *Ictalurus punctatus* and *I. furcatus*) (Darnell, 1961; Millikin and Williams, 1984). A further natural controlling agent is represented by the parasitic dinoflagellate *Hematodinium* sp., known to determine high mortalities in natural populations (Newman and Johnson, 1975; Messick and Shields, 2000; Pagenkopp Lohan *et al*., 2012). In European waters, the pathogen has been described in a number of crustacean species, including brachyurans (Small *et al*., 2012 and literature cited; Small, 2012), no information are available regarding its association with *C. sapidus* in the Mediterranean Sea yet.

In the present study, we surveyed the occurrence of *C. sapidus* in two coastal habitats located on the Adriatic and Ionian coasts of the Salento peninsula (Southern Italy). The survey was carried out over a whole year using crab traps, allowing a previously unattempted quantitative estimation of seasonal variations in the species abundance in Mediterranean coastal habitats. In addition, we screened the hemolymph of sampled crab specimens in order to verify the occurrence of dinoflagellates belonging to the genus *Hematodinium* and the species-specificity of the parasite-host interaction.

Materials and Methods

Study sites

The study was carried out in two transitional water ecosystems of the South Apulia coastline (Salento Peninsula, SE Italy) i.e., the Acquatina lagoon and the Torre Colimena basin. The Acquatina lagoon (40°27’22”N, 18°12’24’’E; Fig. 1) is a semi-artificial transitional water ecosystem created in the 1930s located on the Adriatic Sea. Information on the system morphology and oceanography is provided in Belmonte (2009), Mancinelli (2012), and Mancinelli *et al*. (2013). The lagoon is a shallow (1.2 m average depth) coastal basin with a surface area of 0.45 km^2 and about 2 km long. Dense stands of the seagrass *Cymodocea nodosa* (Ucria) Ascherson cover more than 50% of the bottoms, while other seagrasses of the genera *Ruppia* and *Zostera* together with macroalgae of the genera *Ulva*, *Ceramium*, *Chondria* and *Caulerpa* are locally abundant (Petrocelli and Cecere, 2009).

The benthic macrofauna and macroflora of the Acquatina lagoon have been extensively studied (De Mitri, 2004; Lumare *et al*., 2009a, 2009b; Schirosi *et al*., 2010; Mancinelli, 2012) and they are similar to other Adriatic transitional water ecosystems (Mancinelli and Rossi, 2001; Menéndez *et al*., 2003; Mancinelli *et al*., 2005; Mancinelli *et al*., 2007a, 2007b; Orfanidis *et al*., 2008; Ponti *et al*., 2008; Basset *et al*., 2012; Pinna *et al*., 2013). Large-sized decapods are mainly represented by brachyuran species such as *Carcinus mediterraneus*, Czerniavsky

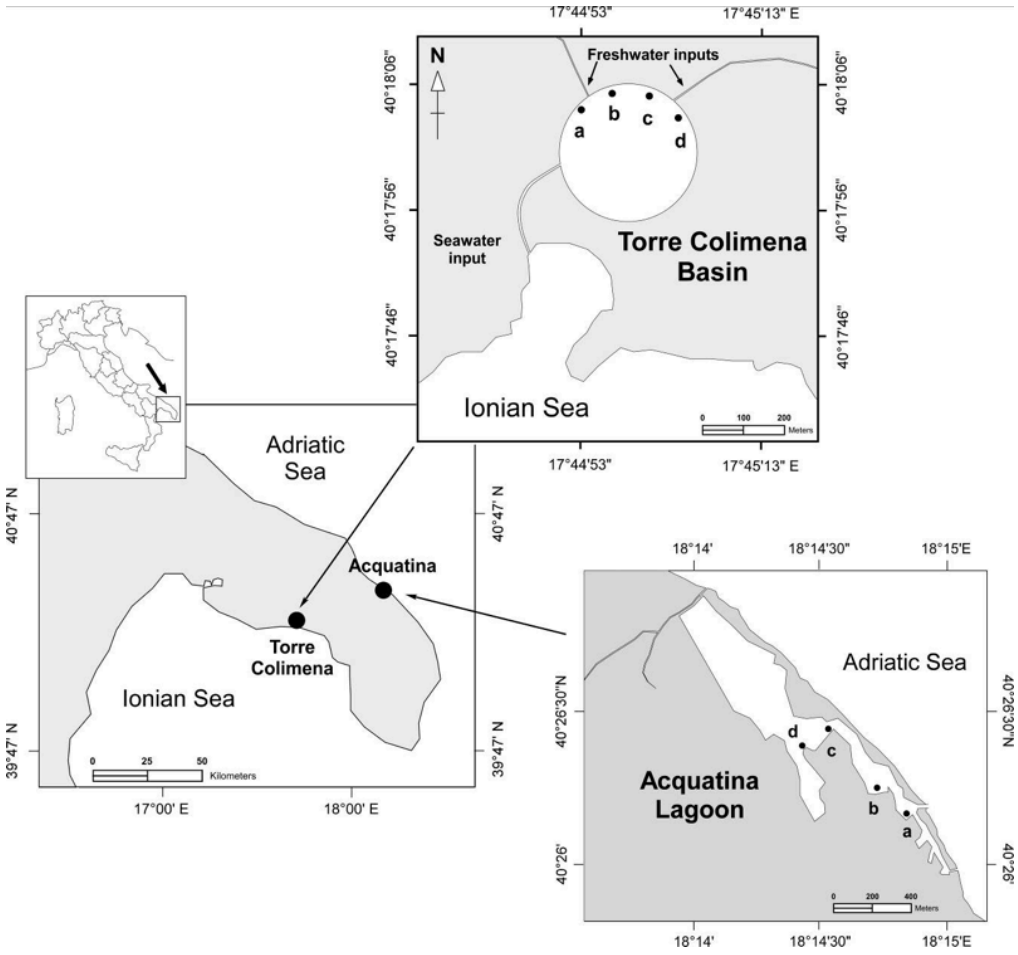

Figure 1. Location of the Torre Colimena basin and Acquatina lagoon in the Apulia coastline (SE Italy). Sampling stations are indicated by black circles and consecutive letters.

(Portunidae), *Eriphia verrucosa*, Forskal (Xantidae) and *Pachygrapsus marmoratus*, Fabricius (Grapsidae). *Maja verrucosa*, Milne-Edwards (Majidae) occurs episodically, while the hermit crab *Pagurus anachoretus*, Leach (Diogenidae) can be locally common. Detailed information on the Torre Colimena basin are provided in Carrozzo (2012) and in Carrozzo *et al*. (2013); in brief, the basin is a shallow (1.2 m average depth) artificial water body located along the Ionian coasts of the Salento Peninsula (40°17'59"N; 17°44'57"E), with a surface area of 0.1 km^2 and about 0.35 km in diameter. It receives freshwater inputs from two drainage ditches, while a channel connects the basin with the Ionian Sea (Fig. 1). The seagrass *C. nodosa* characterizes the soft bottom of the basin, while other seagrasses of the genera *Zostera* and *Ruppia* and macroalgae of the genera *Caulerpa* and *Chondria* are locally abundant. The benthic macrofauna is a brackish-water assemblage typical of other lagoons and coastal habitats of the Salento peninsula (e.g., Alemanno *et al*., 2007a, 2007b, 2007c; Galuppo *et al*., 2007; Mancinelli et al., 2005, 2007a, 2008, 2009; Potenza and Mancinelli, 2010; Mancinelli, 2010; Vignes *et al*., 2012). Among autochthonous brachyuran crustaceans, *C. mediterraneus* and *P.*

marmoratus are commonly observed along the banks of the basin.

Sampling procedures

Crabs were sampled with modified commercial crab traps (60 x 60 x 60 cm) of the type described by Sturdivant and Clark (2011). In brief, each trap was constructed of vinyl-coated 2 × 2 cm mesh wire with an upper and lower chamber. A 15 cm wide entrance was located on each of the four sides of the lower chamber, and a conical bait well was situated in the centre. The upper chamber was the parlour or trap chamber. The two chambers were separated by a wire-mesh panel, raised in the middle to form an inverted V. There were two openings along the apex of the V leading into the parlour. Each trap was attached to a line with a float for retrieval.

In April 2012, four sampling stations were located in each of the study habitats (Fig. 1). One crab trap was located at each sampling station at a depth of 70 - 100 cm. Each trap was baited with fish carcasses and fragmented mussels. Traps were deployed between 6.00 and 7.00 p.m. and retrieved the day after between 9.00 and 10.00 a.m. Crabs were harvested and transferred to the laboratory in refrigerated containers. Sampling operations were repeated adopting an identical procedure in summer (June), autumn (September), and winter (December).

Laboratory analyses

At each sampling time, captured crabs were identified and enumerated. Consequently, crab abundance was expressed in terms of catch per unit effort (CPUE hereafter) calculated as number of individuals per trap. Consequently, the hemolymph was extracted from each specimen from the arthrodial membrane at the juncture of the basis and ischium of one of the swimming legs using a disposable 1ml syringe coupled to a 25-G needle and containing an anti-coagulant solution (0.3 M NaCl, 0.1 M glucose, 30 mM sodium citrate, 26 mM citric acid, 10 mM EDTA, pH 5.4). One drop of hemolymph was mixed (1:1) with neutral red solution (0.25% w/v in filtered lagoon water) and examined under a light microscope. Parasites cells were stained by the solution and appeared red while host hemocytes remained translucent.

Results

Occurrence of Callinectes sapidus

In total, 70 *C. sapidus* specimens were collected during the study period; 53 of these were captured in the Torre Colimena basin. In this habitat, no specimens belonging to other crab species were collected, even though during sampling operations both *P. marmoratus* Fabricius and *Carcinus* sp. were observed along the banks in crevices or other refuges. The blue crab was collected throughout the whole study period (Fig. 2); noticeably, peak abundance was observed in summer, while the occurrence of the species decreased considerably in winter and spring seasons.

In contrast, the 17 specimens of *C. sapidus* captured in the Acquatina Lagoon were sampled only in summer and autumn (Fig. 2), with maximal abundance approximately 50% lower than those observed in the Torre Colimena basin. A total of 20 specimens belonging to three other autochthonous brachyuran species were captured in crab traps during the study period at Acquatina lagoon, i.e., *E. verrucosa*, *P. marmoratus* and *C. mediterraneus*. Specifically, *E. verrucosa* was captured in summer and in autumn together with *C. sapidus*, while *P. marmoratus* and *C. mediterraneus* occurred in winter and spring (Fig. 2).

Occurrence of the parasite Hematodinium sp.

Overall, 70.4% of the crab specimens

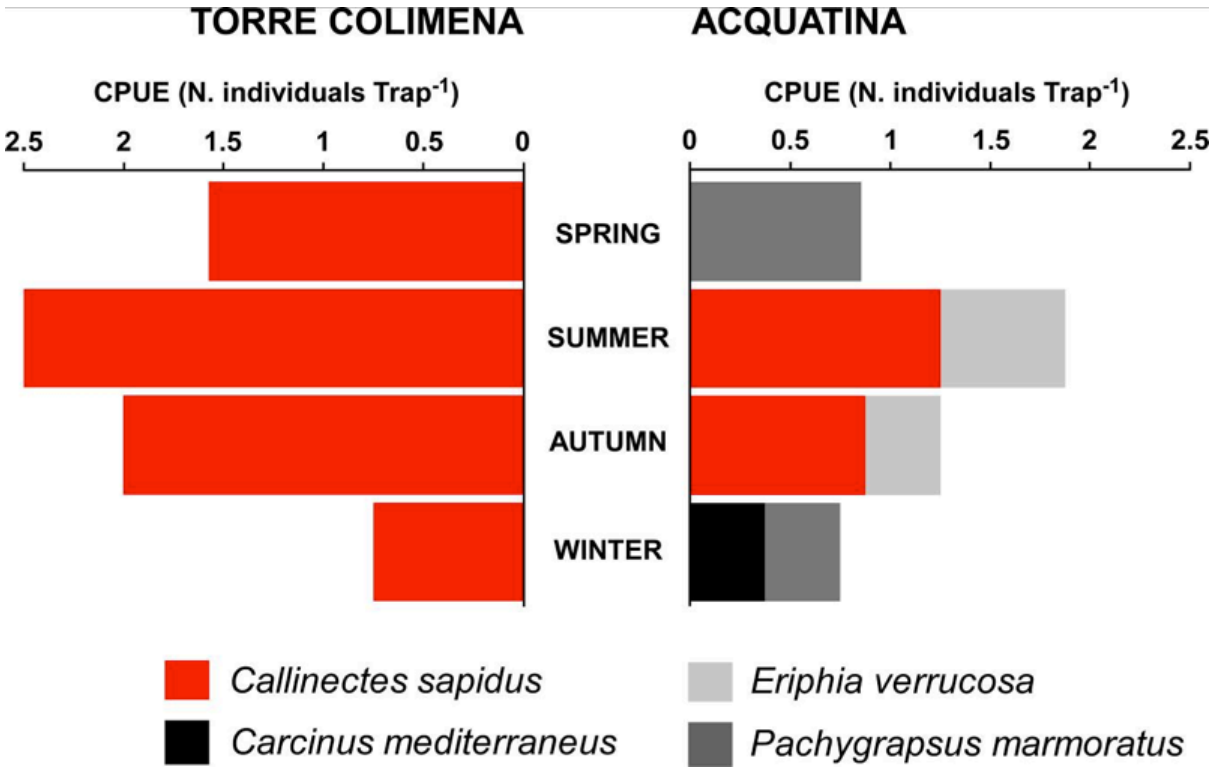

Figure 2. Seasonal patterns of variation in the abundance (expressed as CPUE, catch per unit effort: Individuals Trap-1) of *Callinectes sapidus* in the Torre Colimena basin and Acquatina lagoon during the study period.

examined were infected by the parasitic dinoflagellate *Hematodinium* sp. (Fig. 3). *Callinectes sapidus* resulted more infected than autochthonous crab species (71.6% *vs*. 57.1%) even though the difference was not statistically significant (Difference test for proportions, $P > 0.05$). Blue crabs from Acquatina Lagoon showed a lower degree of infection than conspecifics from Torre Colimena basin (74.1% *vs*. 62.5%), in turn more infected than autochthonous brachyurans (57.1%).

Discussion

The abundance of the blue crab *C. sapidus* was characterized in the two habitats under analysis by different seasonal patterns: while in the Acquatina lagoon it occurred exclusively in summer and autumn months, in the Torre Colimena basin it was captured in crab traps throughout the year. A wide spectrum of biotic and abiotic factors may have determined the observed inter-site differences in the occurrence of *C. sapidus*; among others, temperature may have played a crucial role, since the Torre Colimena basin compared to the Acquatina lagoon was characterized during the study by warmer mean (23.35 ± 1.15 °C *vs*. 21.74 ± 1.08 °C, respectively; mean ± SE) and winter water temperatures (12.21 ± 0.56 °C *vs*. 9.25 ± 0.41 °C, respectively; mean ± SE). In its native habitats, *C. sapidus* is characterized by a period of quiescence in the cold season, overwintering buried in bottom sediments (Millikin and Williams, 1984; Kennedy and Cronin, 2007). The low water temperatures characterizing the Acquatina Lagoon and, in

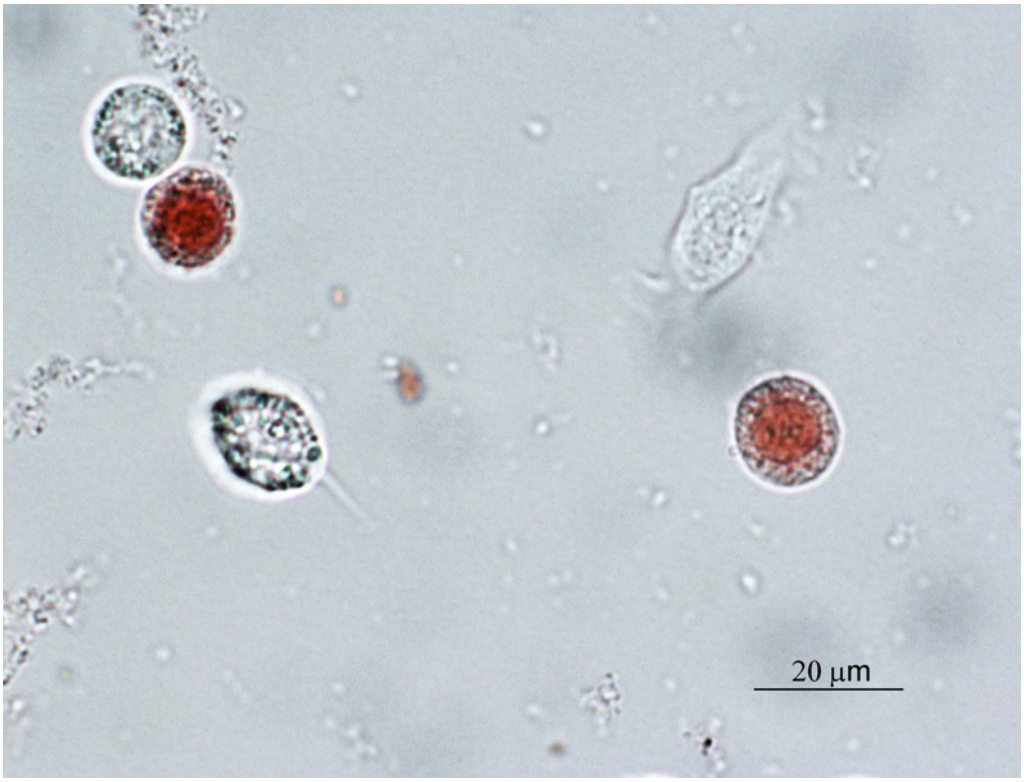

Figure 3. Light micrographs of *Hematodinium* spp. infection in *Callinectes sapidus* hemolymph. Parasites (uninucleate trophonts) are stained by neutral red. Scale bar = 20 µm.

general, the southern Adriatic Sea may have replicated these environmental conditions; in contrast, the warmer Ionian waters are likely to have allowed the blue crab to extend its period of activity, bypassing the winter quiescence.

The abundance patterns observed for other autochthonous brachyurans indirectly suggest that negative interactions may occur between *C. sapidus* and native crab species, in particular those characterized by smaller sizes such as *P. marmoratus* and *C. mediterraneus*. The large-sized *Eriphia verrucosa*, conversely, seems to be less affected by the occurrence of the blue crab in the Acquatina Lagoon. In addition, it is apparent that in the Torre Colimena basin - characterized by a regular occurrence of the blue crab at high abundances - autochthonous crab species, even though present in the benthic environment as testified by qualitative observations, did not occur in crab traps.

Indeed, strong interspecific competition has been observed between native and non-indigenous crab species in western Atlantic and eastern Pacific shore systems (McDonald *et al.*, 2001; Jensen *et al.*, 2002). Here, body size seems to influence the occurrence and magnitude of the inter-specific interaction directly, or mediated by differential response related to species-specific variations in metabolism and, in turn, sensitivity to temperature fluctuations in the benthic environment. Further field and laboratory studies are needed to assess the nature and strength of the interactions (competition for trophic resources, or intra guild predation) linking the blue crab to other native brachyurans or, alternatively, the

influence of abiotic constraints in regulating the abundance and co-occurrence of crab species.

The second and most apparent finding of the present study is represented by the high frequency of the infection by the parasite *Hematodinium* sp. in the blue crab hemolymph, independently from the habitat, and the occurrence of the parasite in autochthonous brachyurans. Species of the genus *Hematodinium* are fast reproducing parasitic alveolates belonging to the class Syndinea. Within weeks from the first signs of infection by *Hematodinium*, the hemolymph of host crustaceans turns from opalescent to milky-white as parasite cells fill the hemal sinuses and replace crustacean hemocytes; muscle tissues of infected animals are degraded by the parasite, the hosts metabolic functions are severely compromised and death occurs within weeks of infection ("bitter crab syndrome", BCS hereafter; Field *et al*., 1992; Stentiford and Shields, 2005). In the United States and Canada, the BCS has raised considerable concern for commercially valuable crustacean species (Stentiford and Shields, 2005; Shields, 2012; Stentiford *et al*., 2012b), as epizootics of *Hematodinium* spp. have severely affected a number of important fisheries. For example, in Alaskan tanner crabs (*Chionoecetes bairdi*) the parasite occurred in a third of fished districts, causing substantial decline in crab populations (Meyers *et al*., 1990); similarly, the BCS has been recognized as one of the causes of the dramatic decline of the snow crab *Chionoecetes opilio* (Taylor and Khan, 1995; Pestal *et al*., 2003). Most importantly, the *C. sapidus* fishery has suffered significant annual mortalities in high-salinity waters of Maryland and Virginia (Messick, 1994; Messick and Shields, 2000).

In European waters, infections have been observed in Scotland, where populations of *Nephrops norvegicus* have experienced *Hematodinium* infection rates of up to 70% (Field *et al*., 1992). In addition, the French velvet crab *Necora puber* fishery suffered a catastrophic reduction due to *H. perezi* (Wilhelm and Mialhe, 1996), and the edible crab *Cancer pagurus* fishery was also affected by *Hematodinium* spp. pathogens (Ní Chualáin *et al*., 2009). *Hematodinium* parasites have been observed also in the caridean *Crangon crangon* (Stentiford *et al*., 2012a).

In the Mediterranean Sea the parasite has been reported only episodically (Stentiford and Shields, 2005; Small *et al*., 2012) while no information are to date available for Italian waters beside the present study. Here, the presence of *Hematodinium* in the hemolymph of *C. sapidus* and autochthonous brachyurans such as *C. mediterraneus*, *E. verrucosa* and *P. marmoratus* clearly indicates that the parasite may be a host generalist, a point elaborated in recent studies (Eigemann *et al*., 2010; Pagenkopp Lohan *et al*., 2012).

The importance of parasites in regulating animal invasions is widely acknowledged (Prenter *et al*., 2004; Hatcher *et al*., 2006; Wood *et al*., 2007; Hatcher *et al*., 2008; Lefèvre *et al*., 2009; Dick *et al*., 2010), and a huge spectrum of alternative hypotheses can be considered regarding the respective roles played by the blue crab, the native brachyuran species and the pathogen as preferential hosts, reservoir hosts and transmission routes. These alternative scenarios are briefly summarized in figure 4, ranging from apparent competition (A) to intra-guild predation (G). The information available to date do not allow to discriminate unambiguously among them; further efforts are needed to verify whether different species of *Hematodinium* occur among crab species, and the species-specificity of the infection. Only the identification of genetic similarities using PCR-based approaches and the construction of phylogenies of parasites from different host species can recognize potential alternate hosts and transmission

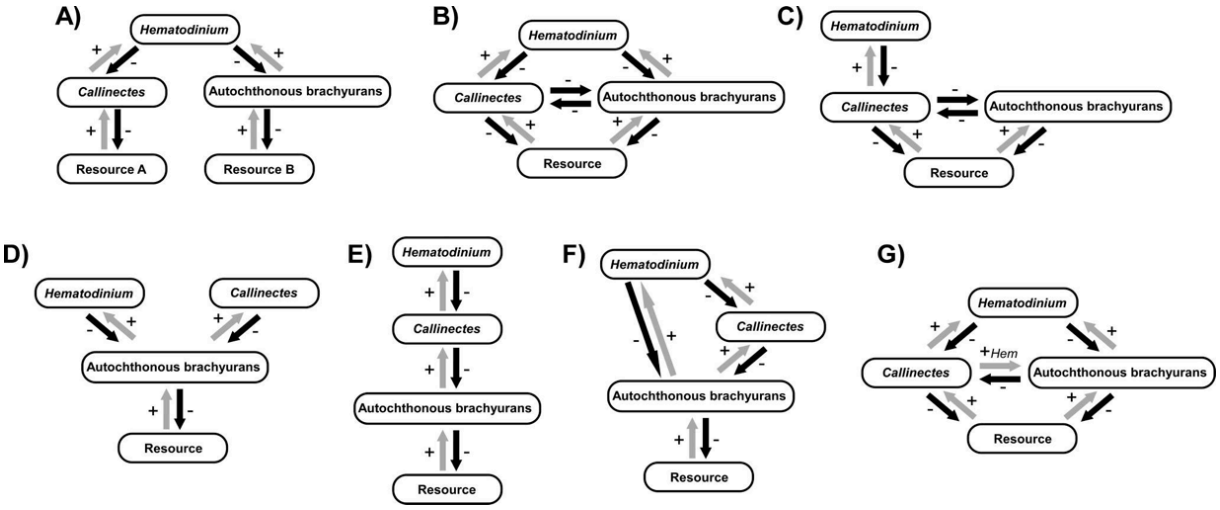

Figure 4. Possible interactions involving parasites: (A) apparent competition; (B) interspecific resource competition with shared parasite (including interference competition as denoted by horizontal arrows); (C) interspecific resource competition and a specialist (not shared) parasite; (D) predator–prey interaction with infected prey (the parasite and predator compete for prey resource); (E) predator–prey interaction with infected predator (conforming to a simple food chain); (F) predator and prey with shared parasite (as may occur for intermediate/definitive host/vector transmission relationships); (G) parasite infecting two intra-guild predators (with *Callinectes* preying more efficiently on native brachyurans). Modified from Hatcher *et al*. (2006).

routes (Archie *et al*., 2009; Nagle *et al*., 2009), ultimately identifying the ecological consequences of the parasite-host interaction in terms of spill-over and spill-back effects (Kelly *et al*., 2009; Pagenkopp Lohan *et al*., 2012).

In conclusion, this study presents novel quantitative information on the co-occurrence of the blue crab *C. sapidus* and its parasite dinoflagellate *Hematodinium* sp. In addition, it suggests an approach to the estimation of the ecological impact of alien species in Mediterranean waters, articulated on the integration of data on the spatial-temporal occurrence of the alien species itself together with information on the host-parasite interactions in which is involved. Ranging from a population to a cellular and molecular scale, the proposed approach has the potential to provide useful information on the mechanisms driving the dispersal and establishment of the blue crab in the Mediterranean, on the effects exerted on autochthonous brachyuran species and, in general, on the biodiversity of benthic systems.

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